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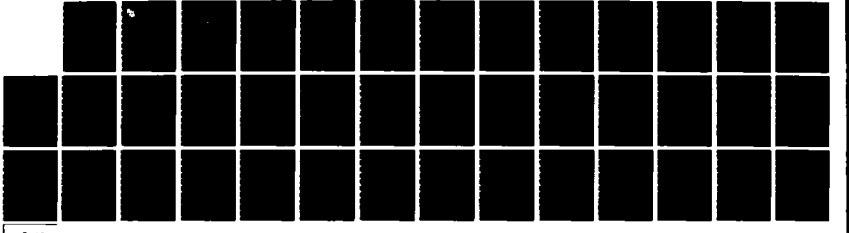

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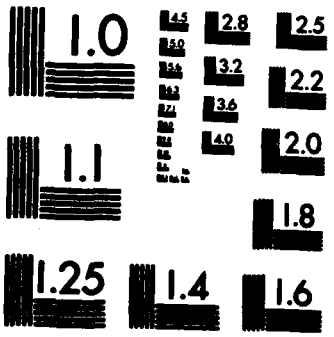

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INSTITUTE REPORT NO. 173

AD-A142 925

DERMAL SENSITIZATION POTENTIAL OF INSECT REPELLENTS: Methyl N,N'-Dihexylethylenediaminemonocarbamate (CHR4), (E)-1,2,3,4-Tetrahydro-6-Methyl-1-(2-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR5), and 1,2,3,4-Tetrahydro-6-Methyl-1-(3-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR6)

THOMAS P. KELLNER, BA, SP5
MARTHA A. HANES, DVM, CPT VC
and
LEONARD J. SAUERS, MS, SP5

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TOXICOLOGY GROUP,
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JUNE 1984

Toxicology Series 62

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

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Dermal Sensitization Potential of Insect Repellents: Methyl N, N'-Dihexylethylenediaminemonocarbamate (CHR4), (E)-1,2,3,4-Tetrahydro-6-Mehtyl-1-(2-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR5), and 1,2,3,4-Tetrahydro-6-Methyl-1-(3-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR6) (Toxicology Series 62)--Kellner, Hanes, and Sauers

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This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. *(AR 360-5)*

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER LAIR Institute Report No. 173	2 GOVT ACCESSION NO. AD-A142 925	3 RECIPIENT'S CATALOG NUMBER
4. TITLE *(and Subtitle)* Dermal Sensitization Potential of Insect Repellents: CHR4, CHR5, and CHR6*		5 TYPE OF REPORT & PERIOD COVERED Final 8 Sep - 25 Oct 82
		6 PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Thomas P. Kellner, BA, SP5 Martha A. Hanes, DVM, CPT VC Leonard J. Sauers, MS, SP5		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Toxicology Group, Division of Research Support Letterman Army Institute of Research Presidio of San Francisco, CA 94129		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS Project 3M161102B74A Work Unit No. 201
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick Frederick, MD 21701		12. REPORT DATE 20 May 84 13. NUMBER OF PAGES 35
14. MONITORING AGENCY NAME & ADDRESS *(if different from Controlling Office)*		15. SECURITY CLASS. *(of this report)* UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

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17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

*CHR4 = Methyl N, N'-dihexylethylenediaminemonocarbamate
CHR5 = (E)-1,2,3,4-Tetrahydro-6-Methyl-1-(2-Methyl-1-Oxo-2-Butenyl) Quinoline
CHR6 = 1,2,3,4-Tetrahydro-6-Methyl-1-(3-Methyl-1-Oxo-2-Butenyl) Quinoline

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Dermal; Sensitization; Insect Repellent: Methyl N, N'-Dihexylethylenediamine-monocarbamate; (E)-1,2,3,4-Tetrahydro-6-Methyl-1-(2 Methyl-1-Oxo-2 Butenyl) Quinoline; 1,2,3,4-Tetrahydro-6-Methyl-1-(3-Methyl-1-Oxo-2 Butenyl) Quinoline

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The insect repellents methyl N,N'-dihexylethylenediaminemonocarbamate (CHR4), (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline (CHR5), and 1,2,3,4-tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl) quinoline (CHR6) were tested for dermal sensitization potential using the Guinea Pig Skin Sensitization Test. The study was conducted in compliance with the Good Laboratory Practice regulations. The data collected was analyzed by the method of the U.S. Army Environmental Hygiene Agency. By this method, none of the repellents tested indicated sensitizing potential.

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ABSTRACT

The insect repellents methyl N,N'-dihexylethylenediamine-monocarbamate (CHR4), (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline (CHR5), and 1,2,3,4-tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl) quinoline (CHR6) were tested for dermal sensitization potential using the Guinea Pig Skin Sensitization Test. The study was conducted in compliance with the Good Laboratory Practice regulations. The data collected was analyzed by the method of the United States Army Environmental Hygiene Agency. By this method, none of the repellents tested indicated sensitizing potential.

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PREFACE

TYPE REPORT: Dermal Sensitization GLP Study Report

TESTING FACILITY: U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Division of Research Support
Presidio of San Francisco, CA 94129

SPONSOR: U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

PROJECT/WORK UNIT/APC: Prevention of Military Disease Hazards
3M16770871, Work Unit 201, APC FL07

GLP STUDY NUMBER: 82025

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC
Diplomate, American College of
Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: CPT Martha A. Hanes, DVM, VC

CO-PRINCIPAL INVESTIGATOR: SP5 Leonard J. Sauers, MS

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of each test compound will be retained in the LAIR Archives.

TEST SUBSTANCES:

A. Methyl N,N'-Dihexylethylenediaminemonocarbamate (CHR4)

B. (E)-1,2,3,4-Tetrahydro-6-Methyl-1-(2-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR5)

C. 1,2,3,4-Tetrahydro-6-Methyl-1-(3-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR6)

INCLUSIVE STUDY DATES: 8 September - 25 October 1982

OBJECTIVE: To evaluate the dermal sensitization potential of insect repellents CHR4, CHR5, and CHR6.

ACKNOWLEDGMENT

The authors wish to thank SP5 Evelyn Zimmerman, SP5 Larry Mullen; BS, and Carolyn M. Lewis; MS, for their assistance in performing the research.

Signatures of Principal Scientists involved
in the Study

We, the undersigned, believe the study number 82025 described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations outlined by the Food and Drug Administration.

Thomas Kellner 18Aug83 for John W Fruin 2MAY84

THOMAS P. KELLNER, BA / DATE
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Author

JOHN T. FRUIN, DVM, Ph.D. / DATE
COL, VC
Study Director

Martha A. Hanes 31Aug83 Leonard J. Sauers 30 June 83

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Co-Principal Investigator

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF

SGRD-ULZ-QA 2 May 84

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 82025 the following inspections were made:

15 Sep 82

1 Oct 82

21 Oct 82

The report and raw data for this study were audited on 10 Apr 84

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the Jan 1983 report to Management and the Study Director.

NELSON R. POWERS, Ph.D.
DAC
Chief, Quality Assurance Unit

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Dermal Sensitization Potential of Insect Repellents: CHR4, CHR5, and CHR6 -- Kellner et al

Letterman Army Institute of Research (LAIR) has been directed to participate in the development of better insect repellents for the protection of soldiers from insects and insect-borne diseases in the field. In the last several years, investigators in the Division of Cutaneous Hazards at LAIR have tested a large number of chemical compounds, submitted by SRI-International, the U.S. Department of Agriculture (USDA); and private industry. These compounds have been tested against a variety of mosquitoes, sand flies, fleas, bugs, ticks, and mites in both in vivo and in vitro test systems. Several compounds have shown sufficient repellent activity and persistence on the skin of animals to warrant consideration for use in lieu of, or in conjunction with, the current troop-issue repellent, 71.25% diethyl-toluamide (m-DEET) in ethanol. The investigators have also evaluated a number of new formulations of m-DEET prepared at LAIR or submitted by private industry. Several of these new formulations have been more persistent on the skin in tests on animals than the current troop-issue repellent.

We now plan to test on human volunteers the most promising of the new compounds and formulations to confirm the results that have been obtained in the experimental studies and evaluate the performance of these agents under conditions of actual use. Before this can be done, it is necessary to obtain certain toxicity data on each compound or formulation to insure that it is safe for application to the skin. The basic toxicity tests required for experimental use of the new compounds and formulations on human volunteers are prescribed by the LAIR and U.S. Army Medical Research and Development Command (USAMRDC) Human Use Committees. If adverse toxicity data are obtained in these tests, the material(s) will be eliminated from consideration, and the prospective tests on human volunteers will not be conducted. The toxicity testing program thereby serves both as an assessment of safety and a secondary screen in the repellent development scheme.

Objective of the Study

The objective of the study is to evaluate the dermal sensitization potential of insect repellents:

Methyl N,N'-Dihexylethylenediaminemonocarbamate (CHR4)

(E)-1,2,3,4-Tetrahydro-6-Methyl-1-(2-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR5)

1,2,3,4-Tetrahydro-6-Methyl-1-(3-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR6)

MATERIALS

Test Substances

1. Chemical name: Methyl-N,N'-Dihexylethylenediamine-monocarbamate (CHR4)

   Chemical Abstract Service Registry No.: None

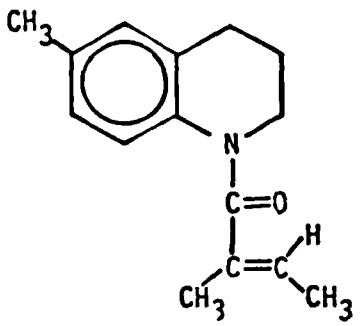
   Structural formula: $CH_3(CH_2)_5NH(CH_2)_2\,N(CO_2CH_3)(CH_2)_5CH_3$

   Empirical formula: $C_{16}H_{34}N_2O_2$

2. Chemical name: 1,2,3,4-Tetrahydro-6-Methyl-1-(2-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR5)

   Chemical Abstract Service Registry No.: None

   Structural formula:

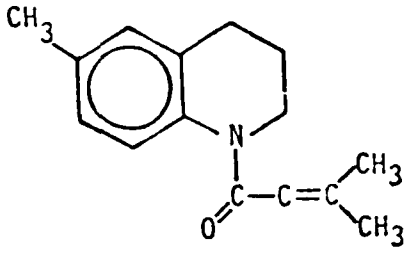


   Empirical formula: $C_{15}H_{19}NO$

3. Chemical Name: 1,2,3,4-Tetrahydro-6-Methyl-1-(3-Methyl-1-Oxo-2-Butenyl) Quinoline (CHR6)

   Chemical Abstract Service Registry No.: None

Structural formula:



Empirical formula: $C_{15}H_{19}NO$

Additional chemical data appear in Appendix A.

Vehicle

The vehicle consisted of two substances, propylene glycol, U.S.P. and 0.9% saline, U.S.P. The propylene glycol (lot no. 36485) was manufactured by Dow Chemical Co. (Freeport, Texas 77541). The sterile saline (lot no. 8C865AH) was manufactured by Travenol Laboratories (Deerfield, Ill. 60015). Propylene glycol and saline have been used successfully as vehicles for this test and were compatible with the test substances.

Animal Data

Fifty male guinea pigs (Hartley strain) were used for the dermal sensitization test. They were acquired from Simonsen Labs, Inc., Gilroy, California. The initial body weight range was 278 to 422 g (mean = 362 g, standard deviation = 32). Each guinea pig was ear tagged (as per LAIR SOP-OP-ARG-1) and their cages were labeled with the corresponding number. Additional animal data appear in Appendix B.

Husbandry

The guinea pigs were housed individually in stainless steel, wire mesh cages with automatic flushes. Their diet consisted of Purina Certified Guinea Pig Chow no. 5025 (lots JUN14821, JUL07822, AUG09821 and AUG11822). Water was provided by automatic lick dispensers connected to a central line. The temperature and relative humidity of the room were constantly monitored and were 71 $\pm$ 3 F (22 $\pm$°1 C) and 50 $\pm$ 5%, respectively. The photoperiod was between 0530 - 2000 hours (light 14.5 hours).

METHODS

Acclimatization and Group Assignment

After one week of quarantine, the guinea pigs were shaved by close-clipping a strip running from the posterior flank to the scapular region on each side and across the back. Animals were assigned to the various treatment groups using the RANDOM program of the Eclipse C330 Computer. Each test group and the positive control group were assigned 10 animals while the positive cage and negative control groups were assigned 5 animals per group.

Compound Preparation

A 3% stock solution of each test substance was prepared by adding 0.3 ml of test substance to 9.7 ml of propylene glycol. Immediately before dosing, 0.5 ml of 3% stock solution was added to 14.5 ml of physiologic saline to give a 0.1% test substance preparation.

Dose Levels

An initial dose of 0.1 ml of 0.1% solution of test substance was injected intradermally in the right scapular area through a 26-gauge needle with a tuberculin syringe (1). Two days later, an injection of 0.1 ml of 0.1% test substance was given. Injections were repeated three times weekly for three weeks, starting in the right lumbosacral area. Similar injections of carrier solution (propylene glycol and saline) were injected at corresponding locations on the left side of the animals' back. Two weeks following the final injection, a challenge dose of 0.1 ml of 0.1% solution of test compound and carrier solution was administered on the right and left sides, respectively.

In addition to the test animals, control groups of guinea pigs were also maintained. A positive control substance, 1-Chloro-2,4-dinitrobenzene (DNCB), was injected into a group of ten guinea pigs on the same dosing schedule as was followed for the test animals. Another group of five guinea pigs, the positive cage controls, received only one 0.1 ml dose of DNCB and this occurred on the challenge dose day. The remaining group of five guinea pigs served as untreated negative controls.

Dates Doses Administered

Appendix C is the historical listing of study events.

Test Procedure

The injection sites (test substance, right side; vehicle control, left side) were scored at 24 and 48 hours after injection. The scoring system was designed so that the intensity of the skin reaction was represented by a proportionate numerical value. The product of the width and length (in millimeters) of the lesion was multiplied by the following reaction scores:

0 = needle puncture
1 = very faint pink - no wheal
2 = faint pink
3 = pink
4 = red
5 = bright red
6 = edema 1 mm in height
7 = edema 1 mm in height
8 = necrosis 1 sq mm
9 = necrosis 1 sq mm

Data Analysis - Phase I

Scores to be utilized in the first phase of data analysis, which were taken 24 and 48 hours after the sensitizing and challenge doses, appear in Tables 1 - 4 of Appendix D. The scores that appear in each 24 and 48 hour column were obtained by subtracting the carrier score from the test substance score. An average score was calculated for each column and grand averages for each dose were obtained by using the 24 and 48 hour average score. These grand averages appear at the bottom of Tables 1 - 4 of Appendix D. Test substance grand averages for the challenge dose were compared to averages obtained for each of the 10 sensitizing doses to determine sensitizing potential. If the value obtained for the challenge dose was substantially higher than averages obtained for ten sensitizing doses, then the substance would be considered a sensitizing agent (2).

Data Analysis - Phase II

The second phase of data analysis was conducted according to the method of the Army Environmental Hygiene Agency (AEHA). This analysis was performed in an attempt to assign a definite value of sensitizing potential to the compounds tested. In this method, a single final grade was obtained for the 24 and 48 hour observations by using the initial dose and the challenge dose scores only. The method of calculation of numerical values from skin reaction scores is shown in Table 1. By this method, a final grade of 25 or less indicates no sensitizing potential (3). The results of this data analysis method are shown in Tables 5-12 of Appendix D. Scores for the 24-hour observation of sensitizing dose number 2 were not recorded. This deviation from the protocol did not affect the data analysis.

TABLE 1

CALCULATION OF NUMERICAL VALUES FROM SKIN REACTION SCORES*

The numerical values of the 24-hour readings are calculated from the following equations:

$G_2 - G_1 = a$

$G_4 - G_3 = b$

$b - a$ = final grade

Where G_1 = 24 hour reaction score from initial injection of vehicle
G_2 = 24 hour reaction score from challenge injection of vehicle
G_3 = 24 hour reaction score from initial injection of test substance
G_4 = 24 hour reaction score from challenge injection of test substance

The numerical values of the 48-hour readings are calculated from the following equations:

$G_6 - G_5 = c$

$G_8 - G_7 = d$

$d - c$ = final grade

Where G_5 = 48 hour reaction score from initial injection of vehicle
G_6 = 48 hour reaction score from challenge injection of vehicle
G_7 = 48 hour reaction score from initial injection of test substance
G_8 = 48 hour reaction score from challenge injection of test substance

A final grade of 25 or less indicates no sensitizing potential and a final grade of 100 indicates a moderate sensitization potential, to guinea pigs.

*The Landsteiner Guinea Pig Sensitization Test, as modified by the Chemical Hygiene Fellowship, Mellon Institute; July 1967.

RESULTS

Analysis Phase I (3)

DNCB (Positive Control)

The grand averages obtained for the sensitizing doses of the positive control, DNCB, showed a sharp increase at the fifth sensitizing dose (Table 1, Appendix D). After this dose the averages rose and fell in a random pattern. The grand average score for the challenge dose, 533.1, was higher than any obtained during the sensitizing doses and was much higher than near zero score obtained for the initial dose.

CHR4

The averages for CHR4 peaked at the sixth dose and then increased and decreased randomly (Table 2, Appendix D). As with the positive control, an increase in the average for the challenge dose over the sensitizing doses was seen. In contrast to DNCB, the grand average score for the challenge dose was only 8.1.

CHR5

The averages obtained for CHR5 peaked at the fifth sensitizing dose and then increased and decreased randomly (Table 3, Appendix D). The average obtained for the challenge dose was lower than six of the averages obtained for sensitizing doses.

CHR6

The averages obtained for CHR6 peaked at the sixth sensitizing dose and then rose and fell randomly (Table 4, Appendix D). An increase in the average for the challenge dose over the sensitizing doses was seen, although this value was again low in contrast to the DNCB average.

Analysis Phase II (AEHA Method)

In an attempt to assign a definite value of sensitizing potential to the compounds tested, a second method of data summary and analysis was performed. This data analysis method was taken from the Guinea Pig Skin Sensitization Test as outlined by the U.S. Army Environmental Hygiene Agency (3). The results of the summary are given in Tables 5 - 12, Appendix D.

Average final grades obtained by this method were under 25 for CHR4, CHR5, and CHR6 on both the 24 and 48 hour observations while the positive control showed grades over 500 (Tables 5 - 12, Appendix D).

DISCUSSION

The ability of three candidate insect repellents to produce contact sensitivity (a form of cell-mediated, delayed hypersensitivity immune response) was investigated in this study. This investigation involved a two phase data summary and analysis portion.

In the first phase, individual average scores from the 10 sensitizing doses were compared to the average score for the challenge dose. A test chemical was judged to be a positive sensitizing agent if it displayed both of the following classic responses (4):

- Stronger reaction to the challenge dose than to any induction dose.

- A sudden and sharp increase in intensity of reactions following the fourth or fifth injection.

The positive control substance, DNCB, elicited the classic skin responses of a strong sensitizing agent. Average skin reaction scores increased from 6.25 to 390.75 between the fourth and fifth sensitizing doses and the average score for the challenge dose, 533.1, was higher than any for the sensitizing doses. Although CHR4 showed a slight jump in scores from 1.2 to 2.3 between the fifth and sixth doses and a challenge dose score (8.1) which exceeded all sensitizing scores, the small magnitude of these scores suggested that it was not a sensitizing agent. The pattern of CHR6 scores also resembled the classic response, but again the magnitude of the scores were extremely low. Neither the pattern nor the magnitude of CHR5 scores resembled the classic response.

By this analysis, CHR5 definitely showed no sensitizing potential. CHR4 and CHR6 data were less conclusive but also suggested little or no sensitizing potential.

The second phase of data analysis was based on the method of the AEHA. The method of calculation of numerical values from skin reaction scores is shown in Table 1. By this method a final grade of 25 or less indicates no sensitizing potential.

None of the repellents tested by the AEHA method indicated sensitizing potential. Final grades of well under 25 were obtained for CHR4, CHR5, and CHR6 on both the 24 and 48 hour observations while the positive control showed grades over 500.

CONCLUSION

In the first phase of analysis, CHR5 showed no sensitizing potential. CHR4 and CHR6 data were less conclusive but also suggested little or no sensitizing potential.

In the second phase, CHR4, CHR5, and CHR6, analyzed by the AEHA method showed no sensitization potential in guinea pigs.

RECOMMENDATION

Toxicity testing of these candidate insect repellents should continue for eventual human use screening.

REFERENCES

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2. Association of Food and Drug Officials of the U.S. Appraisal of the safety of chemicals in foods, drugs and cosmetics, 1959.

3. U.S. Army Environmental Hygiene Agency. Topical hazard evaluation program procedural guide. January, 1982.

4. Griffith, J. Predictive and diagnostic testing for contact sensitization. Toxicol Appl Phar 1969; 3: 90-102.

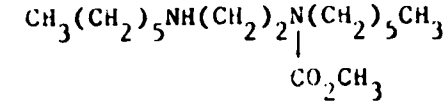
CHEMICAL DATA

CHR4

1. Chemical name: Methyl N,N'-Dihexylethylene-Diaminemonocarbomate

Chemical Abstract Service Registry No.: None

Structural formula:



Empirical formula: $C_{16}H_{34}N_2O_2$

Molecular weight: 286.461

pH: N/A nonaqueous

Physical state: Liquid

Boiling point: 146 C°/ 286.461

Compound density: 0.87 g/ml

Compound refractory index: Unknown

Stability: I.R. spectrum performed by Analytical Chemistry, LAIR, on 4 Aug 82, compared well with I.R. spectrum provided by manufacturer.

Names of contaminants and percentages: Purity data on file with manufacturer.

Manufacturer: Starks Associates, Inc.
Buffalo, NY 14213

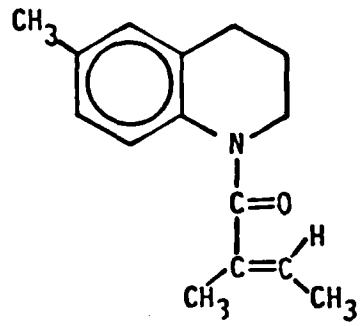
Manufacturer Lot No: 3K38075

CHR5

2. Chemical name: (E) 1,2,3,4-tetrahydro-6-Methyl-1-(2-Methyl-1-Oxo-2-Butenyl)Quinoline

Chemical Abstracts Service Registry No.: None

Structural formula:



Empirical formula: $C_{15}H_{19}NO$

Molecular weight: 229

pH: N/A nonaqueous

Physical state: Solid

Boiling point: Unknown

Compound density: Unknown

Compound refractory index: Unknown

Stability: I.R. spectrum performed by Analytical Chemistry, LAIR, on 3 Jan 84, compared well with I.R. spectrum provided by manufacturer.

Names of contaminants and percentages: Purity data on file with manufacturer.

Manufacturer: Starks Associates, Inc.
Buffalo, NY 14213

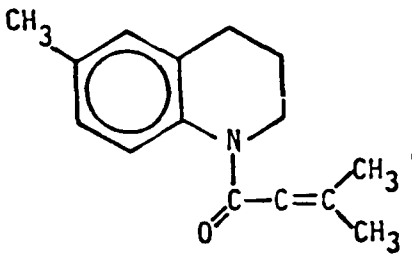
Manufacturer Lot No.: 4214H31

CHR6

3. Chemical Name: 1,2,3,4-Tetrahydro-6-Methyl-1-(3-Methyl-1-Oxo-2-Butenyl)Quinoline

Chemical Abstracts Service Registry No.: None

Molecular structure:



Empirical formula: $C_{15}H_{19}NO$

Molecular weight: 229

pH: N/A nonaqueous

Physical state: Liquid

Boiling range: Unknown

Compound density: Unknown

Compound refractory index: Unknown

Stability: I.R. spectrum performed by Analytical Chemistry, LAIR, on 22 Jun 82, compared well with I.R. spectrum provided by manufacturer.

Names of contaminants and percentages: Purity data on file with manufacturer.

Manufacturer: Starks Associates, Inc.
Buffalo, NY 14213

Manufacturer Lot No.: 3905H3

ANIMAL DATA

Species: Guinea Pig

Strain: Hartley

Source: Simonsen Labs, Inc.
Gilroy, CA

Sex: Male

Age: Young adults

Method of randomization: RANDOM Program on Eclipse C330

Animals in each group:

10 males in each test chemical group
10 males in positive control group
5 males in positive cage control group
5 males in negative control group

Condition of animals at start of study: Normal

Body weight range: 278 - 472 g; mean = 362; standard deviation = 32

Identification procedures: Ear tag (SOP-OP-ARG-1)

Pretest conditioning:

1. Animals were observed for illness, ear tagged, weighed, and housed individually.

2. Animals were close-clipped and randomized into dose groups.

Justification: Guinea pigs are a proven sensitive animal model for this test.

HISTORICAL LISTING OF STUDY EVENTS

Date	Event
8 Sep 82	Animals arrived. They were observed for illness, ear tagged, weighed, and housed individually in GLP suite.
9-14 Sep 82	Animals were observed once daily.
13 Sep 82	Animals were weighed.
14 Sep 82	Animals were close clipped, and randomized into dose groups.
15 Sep 82	Animals were given initial doses, observed 24 hours and 48 hours later.
17, 20, 22, 24, 27, 29 Sep 82 1, 4, 6 Oct 82	Animals were given sensitizing doses, and clipped as needed. Animals were observed 24 and 48 hours after each dose.
20, 27 Sep 82 4, 12, 18 Oct 82	Animals were weighed.
20 Oct 82	Animals received challenge dose.
21 Oct 82	Animals were scored for 24-hour reaction.
22 Oct 82	Animals were scored for 48-hour reaction.
25 Oct 82	Study terminated.

APPENDIX C

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APPENDIX D

TABLE I

GLP STUDY # 82025

GUINEA PIG SENSITIZATION TEST

Chemical Name: DNCB (Positive Control)

Initial & Challenge Date: 15 Sep 82 / 20 Oct 82

Principal Investigator: Hanes

Diluent: Propylene glycol / Saline

Dose #	Initial 1		2		3		4		5		6		7		8		9		10		Challenge	
Animals #	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
82E00012	0	0		0	0	0	0	0	294	180	195	190	540	600	270	54	560	252	300	36	600	400
82E00014	0	0		0	1	4	8	27	384	700	288	500	720	420	700	128	288	120	350	72	294	294
82E00031	0	0		0	0	0	0	18	405	336	45	400	400	400	630	24	225	112	400	54	486	486
82E00038	0	0		0	0	0	0	4	294	288	126	598	168	210	150	45	540	112	300	168	486	486
82E00043	0	0		0	0	0	0	2	293	378	280	420	600	560	770	200	630	180	400	336	600	486
82E00045	-1	0		0	0	0	9	4	600	288	440	300	500	600	480	60	588	336	384	180	880	800
82E00049	0	0		0	0	0	0	27	599	720	480	500	500	600	288	24	600	90	180	36	540	540
82E00056	0	0		0	0	0	0	16	294	0	240	294	200	324	280	60	1200	84	196	100	480	640
82E00062	0	0		0	0	4	0	8	599	600	400	700	405	600	840	252	490	336	224	46	900	560
82E00063	0	0		0	0	0	0	2	383	280	120	100	324	480	324	40	600	162	147	12	384	320
Average Score	-0.1	0	N/A	0	0.1	0.8	1.7	10.8	414.5	377	261.4	400.2	435.7	479.4	473.2	88.7	572.1	178.4	288.1	103	565	501.2

Average Score for Challenge: 533.1

Average Score for
1. -0.05
2. N/A
3. 0.45
4. 6.25
5. 395.75
6. 330.8
7. 457.55
8. 280.95
9. 375.25
10. 195.55

Injection dates:
1. 15 Sep 82
2. 17 Sep 82
3. 20 Sep 82
4. 22 Sep 82
5. 24 Sep 82
6. 27 Sep 82
7. 29 Sep 82
8. 1 Oct 82
9. 4 Oct 82
10. 6 Oct 82

APPENDIX D

APPENDIX D (cont.)

TABLE 2

GLP STUDY # 82025

GUINEA PIG SENSITIZATION TEST

Chemical Name: CHR4

Initial & Challenge Date: 15 Sep 82 / 20 Oct 82

Principal Investigator: Hanes

Diluent: Propylene glycol / Saline

Dose #	Initial 1		2		3		4		5		6		7		8		9		10		Challenge	
Animals #	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	43hr	24hr	48hr	24hr	48hr
82E00015	0	0		0	0	0	0	0	0	0	0	1	4	0	0	0	0	0	50	0	40	25
82E00022	0	0		0	0	0	0	0	8	0	6	0	4	0	0	0	0	0	0	0	15	0
82E00028	0	0		0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	32	1	0	0
82E00029	0	0		0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	1	0	0	3
82E00030	0	0		0	0	0	0	0	2	0	8	2	3	0	0	1	0	0	1	0	4	0
82E00036	1	0		0	0	0	0	0	0	0	11	3	1	0	0	1	0	0	0	0	0	0
82E00040	0	0		0	0	0	0	2	4	0	4	8	0	0	0	0	0	0	0	0	9	1
82E00046	1	0		0	0	0	0	1	-8	0	0	0	4	0	0	0	0	0	0	0	12	0
82E00053	0	0		0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	4	1	32	8
82E00061	0	0		1	0	0	0	0	12	0	0	2	1	0	0	0	0	0	0	0	9	4
Average Score	0.2	0	N/A	0.1	0	0	0	0.4	2.4	0	2.9	1.6	2.0	0	0	0.2	0	0	8.8	0.2	12.1	4.1

Average Score for Challenge: 8.1

Average Score for 1. 0.1 6. 2.3
2. N/A 7. 1.0
3. 0 8. 0.1
4. 0.2 9. 0
5. 1.2 10. 5

Injection dates: 1. 15 Sep 82 6. 27 Sep 82
2. 17 Sep 82 7. 29 Sep 82
3. 20 Sep 82 8. 1 Oct 82
4. 22 Sep 82 9. 4 Oct 82
5. 24 Sep 82 10. 6 Oct 82

TABLE 3

GLP STUDY # 82025

GUINEA PIG SENSITIZATION TEST

Chemical Name: CHR5

Initial & Challenge Date: 15 Sep 82 / 20 Oct 82

Principal Investigator: Hanes

Diluent: Propylene glycol / Saline

Dose #	Initial 1		2		3		4		5		6		7		8		9		10		Challenge	
Animals #	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
82E00011	0	1		1	0	0	0	6	45	1	12	10	44	0	0	0	18	30	50	[illegible]	[illegible]	0
82E00020	0	0		4.5	1	6	1	36	48	12	18	70	42	6	[illegible]	[illegible]	[illegible]	1	0	[illegible]	[illegible]	[illegible]
82E00023	0	0		2	1	0	0	32	67	24	24	8	29	1	[illegible]	[illegible]	[illegible]	[illegible]	18	[illegible]	[illegible]	[illegible]
82E00024	0	0		3	0	4	1	8	50	6	16	4	32	1	0	0	27	1	0	0	[illegible]	[illegible]
82E00025	0	0		6.75	0	8	1	24	12	15	8	1	6	0	3	3	10	0	18	1	[illegible]	[illegible]
82E00034	0	0		2	0	0	0	11	8	4	0	2	4	0	3	1	17	0	0	0	6	1
82E00039	0	0		2	1	0	0	18	12	4	12	39	18	0	8	0	29	4	16	4	16	4
82E00051	0	0		0	0	0	4	64	31	1	30	8	11	0	[illegible]	[illegible]	[illegible]	0	[illegible]	[illegible]	[illegible]	[illegible]
82E00052	1	0		0	0	0	0	4	12	1	0	0	3	0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
82E00054	0	0		4	1	0	0	32	4	48	18	0	8	0	1	1	[illegible]	1	[illegible]	[illegible]	[illegible]	[illegible]
Average Score	0.1	0.1	N/A	2.5	0.4	1.8	0.7	23.5	28.9	11.6	13.8	14.2	19.7	0.8	2.1	1.3	11.4	3.7	15.0	[illegible]	[illegible]	[illegible]

Average Score for Challenge: 5.35

Average Score for 1. 0.10 6. 14.00
2. N/A 7. 10.25
3. 1.10 8. 1.70
4. 12.10 9. 7.55
5. 20.25 10. 8.45

Injection dates: 1. 15 Sep 82 6. 27 Sep 82
2. 17 Sep 82 7. 29 Sep 82
3. 20 Sep 82 8. 1 Oct 82
4. 22 Sep 82 9. 4 Oct 82
5. 24 Sep 82 10. 6 Oct 82

APPENDIX D (cont.)

APPENDIX D (cont.)

TABLE 4

GLP STUDY # 82025

GUINEA PIG SENSITIZATION TEST

Chemical Name: CHR6

Initial & Challenge Date: 15 Sep 82 / 20 Oct 82

Principal Investigator: Hanes

Diluent: Propylene glycol / Saline

Dose #	Initial 1		2		3		4		5		6		7		8		9		10		Challenge	
Animals #	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
82E00013	0	0		0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0
82E00017	0	0		0	0	0	0	0	-2	0	12	0	4	0	0	0	-1	1	4	0	1	0
82E00026	0	0		0	0	0	0	0	0	0	0	0	0	0	4	2	0	0	0	0	0	0
82E00032	0	0		0	1	0	4	4	0	0	0	0	4	6	1	0	0	0	1	0	4	4
82E00035	0	0		0	0	0	0	0	32	0	4	1	0	0	0	0	0	0	1	0	9	4
82E00047	0	0		0	0	0	0	0	-4	0	0	0	1	0	0	0	0	0	0	0	18	0
82E00048	0	0		0	0	0	1	1	0	0	4	0	0	0	0	0	0	0	0	0	0	0
82E00058	0	0		0	0	0	0	1	0	0	12	0	1	0	0	0	0	0	0	0	16	0
82E00059	0	0		2	1	0	1	0	4	0	0	0	0	0	0	0	0	0	0	0	18	8
82E00064	-1	0		0	0	0	1	0	0	0	8	0	1	0	0	0	0	0	0	0	4	2
Average Score	-0.1	0	N/A	0.2	0.2	0	0.7	0.6	3.0	0	4.0	0.1	1.1	0.6	0.9	0.2	-0.1	0.1	0.6	0	7.0	1.8

Average Score for Challenge: 4.4

Average Score for 1. -0.05 6. 2.05
2. N/A 7. 0.85
3. 0.10 8. 0.55
4. 0.65 9. 0.00
5. 1.50 10. 0.30

Injection dates: 1. 15 Sep 82 6. 27 Sep 82
2. 17 Sep 82 7. 29 Sep 82
3. 20 Sep 82 8. 1 Oct 82
4. 22 Sep 82 9. 4 Oct 82
5. 24 Sep 82 10. 6 Oct 82

TABLE 5

CALCULATION OF NUMERICAL VALUES FROM SKIN REACTION SCORES

Study No. 82025 | Test Substance DNCB | 24 Hour Score

Animal Number	Diluent Substance			Test Substance			Final Grade b - a
	G_1	G_2	$G_2{}^{a}-G_1$	G_3	G_4	$G_4{}^{b}-G_3$	
82E000 12	0	0	0	0	600	600	600
82E000 14	0	0	0	0	294	294	294
82E000 31	0	0	0	0	486	486	486
82E000 38	0	0	0	0	486	486	486
82E000 43	0	0	0	0	600	600	600
82E000 45	1	0	-1	0	880	880	881
82E000 49	0	0	0	0	540	540	540
82E000 56	0	0	0	0	480	480	480
82E000 62	0	0	0	0	900	900	900
82E000 63	0	0	0	0	384	384	384
							$\bar{x}$ = 565

G_1 = Score of Initial Dose G_3 = Score of Initial Dose $\bar{x}$ = Average Final Grade

G_2 = Score of Challenge Dose G_4 = Score of Challenge Dose

APPENDIX D (cont.)

APPENDIX D (cont.)

TABLE 6

CALCULATION OF NUMERICAL VALUES FROM SKIN REACTION SCORES

Study No. 82025 Test Substance DNCB 48 Hour Score

Animal Number	Diluent Substance			Test Substance			Final Grade	
	G_5	G_6	$G_6{}^c - G_5$	G_7	G_8	$G_8{}^d - G_7$	d - c	
82E000 12	0	0	0	0	400	400	400	
82E000 14	0	0	0	0	294	294	294	
82E000 31	0	0	0	0	486	486	486	
82E000 38	0	0	0	0	486	486	486	
82E000 43	0	0	0	0	486	486	486	
82E000 45	0	0	0	0	800	800	800	
82E000 49	0	0	0	0	540	540	540	
82E000 56	0	0	0	0	640	640	640	
82E000 62	0	0	0	0	560	560	560	
82E000 63	0	0	0	0	320	320	320	
							$\bar{x}$ = 501	

G_5 = Score of Initial Dose G_7 = Score of Initial Dose $\bar{x}$ = Average Final Grade

G_6 = Score of Challenge Dose G_8 = Score of Challenge Dose

TABLE 7

CALCULATION OF NUMERICAL VALUES FROM SKIN REACTION SCORES

Study No. 82025 Test Substance CHR4 24 Hour Score

Animal Number	Diluent Substance			Test Substance			Final Grade b - a
	G_1	G_2	$G_2{}^a - G_1$	G_3	G_4	$G_4{}^b - G_3$	
82E000 15	0	0	0	0	40	40	40
82E000 22	0	0	0	0	15	15	15
82E000 28	0	0	0	0	0	0	0
82E000 29	0	0	0	0	0	0	0
82E000 30	0	0	0	0	4	4	4
82E000 36	0	0	0	1	0	-1	-1
82E000 40	0	0	0	0	9	9	9
82E000 46	0	0	0	1	12	11	11
82E000 53	0	4	4	0	36	36	32
82E000 61	0	0	0	0	9	9	9
							$\bar{x} = 12$

G_1 = Score of Initial Dose G_3 = Score of Initial Dose $\bar{x}$ = Average Final Grade

G_2 = Score of Challenge Dose G_4 = Score of Challenge Dose

APPENDIX D (cont.)

TABLE 8
CALCULATION OF NUMERICAL VALUES FROM SKIN REACTION SCORES

Study No. 82025 Test Substance CHR4 48 Hour Score

Animal Number	Diluent Substance G_5	G_6	$G_6^c - G_5$	Test Substance G_7	G_8	$G_8^d - G_7$	Final Grade d - c	
82E000 15	0	0	0	0	25	25	25	
82E000 22	0	0	0	0	0	0	0	
82E000 28	0	0	0	0	0	0	0	
82E000 29	0	0	0	0	3	3	3	
82E000 30	0	0	0	0	0	0	0	
82E000 36	0	0	0	0	0	0	0	
82E000 40	0	0	0	0	1	1	1	
82E000 46	0	0	0	0	0	0	0	
82E000 53	0	0	0	0	8	8	8	
82E000 61	0	0	0	0	4	4	4	
							$\bar{x} = 4$	

G_5 = Score of Initial Dose G_7 = Score of Initial Dose $\bar{x}$ = Average Final Grade

G_6 = Score of Challenge Dose G_8 = Score of Challenge Dose

APPENDIX D (cont.)

TABLE 9
CALCULATION OF NUMERICAL VALUES FROM SKIN REACTION SCORES

Study No. 82025 Test Substance CHR4 24 Hour Score

Animal Number	Diluent Substance G_1	Diluent Substance G_2	Diluent Substance $G_2^a - G_1$	Test Substance G_3	Test Substance G_4	Test Substance $G_4^b - G_3$	Final Grade b - a
82E000 11	0	0	0	0	0	0	0
82E000 20	0	0	0	0	0	0	0
82E000 23	0	0	0	0	9	9	9
82E000 24	0	0	0	0	4	4	4
82E000 25	0	0	0	0	0	0	0
82E000 34	0	0	0	0	6	6	6
82E000 39	0	0	0	0	16	16	16
82E000 51	0	0	0	0	50	50	50
82E000 52	0	0	0	1	0	-1	-1
82E000 54	1	0	-1	1	0	-1	0
							$\bar{x} = 8$

G_1 = Score of Initial Dose G_3 = Score of Initial Dose $\bar{x}$ = Average Final Grade

G_2 = Score of Challenge Dose G_4 = Score of Challenge Dose

APPENDIX D (cont.)

APPENDIX D (cont.)

TABLE 10

CALCULATION OF NUMERICAL VALUES FROM SKIN REACTION SCORES

Study No. 82025 Test Substance CHR5 48 Hour Score

Animal Number	Diluent Substance			Test Substance			Final Grade $d - c$	
	G_5	G_6	$G_6{}^c - G_5$	G_7	G_8	$G_8{}^d - G_7$		
82E000 11	0	0	0	1	0	-1	-1	
82E000 12	0	0	0	0	0	0	0	
82E000 23	0	0	0	0	9	9	9	
82E000 24	0	0	0	0	0	0	0	
82E000 25	0	0	0	0	0	0	0	
82E000 34	0	0	0	0	1	1	1	
82E000 39	0	0	0	0	4	4	4	
82E000 51	0	0	0	0	8	8	8	
82E000 52	0	0	0	0	0	0	0	
82E000 54	0	0	0	0	0	0	0	
							$\bar{x} = 2$	

G_5 = Score of Initial Dose G_7 = Score of Initial Dose $\bar{x}$ = Average Final Grade

G_6 = Score of Challenge Dose G_8 = Score of Challenge Dose

TABLE 11

CALCULATION OF NUMERICAL VALUES FROM SKIN REACTION SCORES

Study No. 82025 Test Substance CHR6 24 Hour Score

Animal Number	Diluent Substance			Test Substance			Final Grade b - a
	G_1	G_2	$G_2{}^a - G_1$	G_3	G_4	$G_4{}^b - G_3$	
82E000 13	0	0	0	0	0	0	0
82E000 17	0	0	0	0	1	1	1
82E000 26	0	0	0	0	0	0	0
82E000 32	0	0	0	0	4	4	4
82E000 35	0	0	0	0	9	9	9
82E000 47	0	0	0	0	18	18	18
82E000 48	0	0	0	0	0	0	0
82E000 58	0	0	0	0	16	16	16
82E000 59	0	0	0	0	18	18	18
82E000 64	1	0	-1	0	4	4	5
							$\bar{x} = 7$

G_1 = Score of Initial Dose G_3 = Score of Initial Dose $\bar{x}$ = Average Final Grade

G_2 = Score of Challenge Dose G_4 = Score of Challenge Dose

APPENDIX D (cont.)

TABLE 12

CALCULATION OF NUMERICAL VALUES FROM SKIN REACTION SCORES

Study No. 82025 Test Substance CHR6 48 Hour Score

Animal Number	Diluent Substance			Test Substance			Final Grade d - c
	G_5	G_6	$G_6{}^{c}-G_5$	G_7	G_8	$G_8{}^{d}-G_7$	
82E000 13	0	0	0	0	0	0	0
82E000 17	0	0	0	0	0	0	0
82E000 26	0	0	0	0	0	0	0
82E000 32	0	0	0	0	4	4	4
82E000 35	0	0	0	0	4	4	4
82E000 47	0	0	0	0	0	0	0
82E000 48	0	0	0	0	0	0	0
82E000 58	0	0	0	0	0	0	0
82E000 59	0	0	0	0	8	8	8
82E000 64	0	0	0	0	2	2	2
							$\bar{x} = 2$

G_5 = Score of Initial Dose G_7 = Score of Initial Dose $\bar{x}$ = Average Final Grade

G_6 = Score of Challenge Dose G_8 = Score of Challenge Dose

APPENDIX D (concluded)

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